# Travel Optimization by Foraging Bumblebees through Readjustments of Traplines after Discovery of New Feeding Locations

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ABSTRACT: Animals collecting resources that replenish over time often visit patches in predictable sequences called traplines. Despite the widespread nature of this strategy, we still know little about how spatial memory develops and guides individuals toward suitable routes. Here, we investigate whether flower visitation sequences by bumblebees *Bombus terrestris* simply reflect the order in which flowers were discovered or whether they result from more complex navigational strategies enabling bees to optimize their foraging routes. We analyzed bee flight movements in an array of four artificial flowers maximizing interfloral distances. Starting from a single patch, we sequentially added three new patches so that if bees visited them in the order in which they originally encountered flowers, they would follow a long (suboptimal) route. Bees' tendency to visit patches in their discovery order decreased with experience. Instead, they optimized their flight distances by rearranging flower visitation sequences. This resulted in the development of a primary route (trapline) and two or three less frequently used secondary routes. Bees consistently used these routes after overnight breaks while occasionally exploring novel possibilities. We discuss how maintaining some level of route flexibility could allow traplining animals to cope with dynamic routing problems, analogous to the well-known traveling salesman problem.

*Keywords:* *Bombus terrestris*, pollination ecology, spatial cognition, trapline foraging, traveling salesman problem.

## Introduction

Animals exploiting resources within fixed areas or territories have a set of navigational memories at their disposal that allow them to encode spatial relationships between features of their environment and orient accurately as they gain experience (reviewed in Dolins and Mitchell 2010).

Navigation can be achieved using different mechanisms—such as directional compasses (e.g., Muheim et al. 2006), path integration (e.g., Müller and Wehner 1988; Chittka et al. 1995*b*), learned landmark sequences (e.g., Chittka et al. 1995*a*; Zhang et al. 1996), or learned motor sequences (e.g., Collett et al. 1993)—and often guides individual movements toward functional, if not optimal, routes. Because the complexity of routing problems increases exponentially with the number of sites individuals have to visit (Lawler et al. 1985), travel optimization becomes particularly challenging for central-place foragers collecting resources from multiple scattered patches.

Bees, for example, are known to repeat foraging circuits, visiting a particular set of flowers in a predictable nonrandom order, referred to as trapline foraging in analogy with trappers checking traps on a regular basis (for a review, see Ohashi and Thomson 2009). This strategy, although first reported in pollinating insects (Heinrich 1976; Gilbert 1980), is widespread among animals and has been described in many vertebrates, such as bats (Lemke 1984; Racey and Swift 1985), birds (Davies and Houston 1981; Gill 1988), primates (Janson 1998; Watts 1998; Noser and Byrne 2010), and rodents (Reid and Reid 2005). In recent years, much attention has been focused on the adaptive value of traplining, highlighting that route fidelity can increase individual foraging performance in different ways, whether by allowing animals to (1) learn locations of the most rewarding resources (Williams and Thomson 1998; Garrison and Gass 1999; Cunningham and Janson 2007), (2) set up optimal visitation schedules in relation to resources replenishment rates (Possingham 1989; Ohashi and Thomson 2005), (3) increase travel speed and accuracy of movements (Ohashi et al. 2007; Saleh and Chittka 2007), and/or (4) outcompete less experienced conspecifics when exploiting overlapping areas (Gill 1988; Ohashi et al. 2008). However, the question of how traplines develop

remains surprisingly unexplored in comparison with other navigational strategies.

As pointed out by Anderson (1983), traplining animals regularly cope with routing problems analogous to the well-known traveling salesman problem in mathematics (finding the shortest multidestination route while visiting each location only once). This problem remains time consuming to solve using extensive computing power and still lacks an efficient general solution (Applegate et al. 2006; Gutin and Punnen 2006). Traplining animals, especially those with small brains such as pollinating insects (Menzel and Giurfa 2001; Chittka and Niven 2009), provide an excellent opportunity to test simple heuristics and unravel functional routing solutions (e.g., Bures et al. 1992; Cramer and Gallistel 1997).

Two laboratory studies recently demonstrated that naive bees foraging in stable floral arrays develop repeatable routes as they gain experience (Ohashi et al. 2007; Saleh and Chittka 2007). Area fidelity allows them to establish long-term spatial memory and may favor the storage of landmark sequences and/or motor commands necessary for accurate orientation (Collett et al. 1993; Menzel et al. 2000). However, these results tell us little about the decision-making rules used by bees to develop stable visitation sequences in relation to the spatial locations of flowers or flower patches. In particular, early descriptions of trapline foraging in pollinating insects suggested that the sequence in which individuals visit plants reflects the order in which they were initially incorporated into the route (the discovery order), even though this typically leads to long suboptimal flight distances (Janzen 1971). Experiments with honeybees demonstrate that they can follow novel shortcuts between familiar feeder locations (Menzel et al. 1998; Menzel et al. 2005), indicating that bees might be able to connect multiple locations in a different sequence from that in which they were originally encountered. However, there is still no empirical evidence for this. Observations of small-scale traplining (within-patch foraging), where travel costs may be negligible, suggest that bees prioritize short moves between nearest-neighbor flowers without attempting to minimize overall flight distances (Ohashi et al. 2007; Saleh and Chittka 2007). However, because bees can forage in patches located several kilometers apart (Osborne et al. 2008; Wikelski et al. 2010) and develop traplines covering areas of at least 300 m$^2$ (Comba 1999), we expect them to attempt to optimize routes at larger spatial scales (between-patch foraging) when the travel costs of traplining may be greatly increased.

In this study, we tested whether the traplines used by experienced bumblebees *Bombus terrestris* are based on the discovery order of flower patches or, alternatively, from more complex navigational strategies that might permit more efficient routes. We also investigated to what extent individuals are consistent in their use of foraging circuits after extended breaks, by observing bees in the same floral array over two successive days.

## Methods

Experiments were carried out in a large flight room (length = 870 cm, width = 730 cm, height = 200 cm) set up in a greenhouse (temperature range: 15°–20°C; photoperiod: 12D : 12L), thus providing bees with ecologically realistic dimensions, to investigate large-scale traplining behaviors (between-patch foraging). Greenhouse windows were obscured with white paint (Leyland, Bristol). Controlled illumination was provided by high-frequency fluorescent lighting (TMS 24F lamps with HF-B 236 TLD [4.3 kHz] ballasts [Philips, The Netherlands] fitted with Activa daylight fluorescent tubes [Osram, Germany]), which simulates natural daylight above the bee flicker fusion frequency. Our subjects were workers from a commercially obtained *Bombus terrestris* colony (Syngenta Bioline Bees, Weert, The Netherlands), housed in a bipartite wooden nest box (length = 28 cm, width = 16 cm, height = 11 cm). The movements of bees from the nest box to the flight room could be carefully controlled using a series of shutters in the transparent entrance tube to the nest box. All workers were marked on their thorax using individually numbered colored tags (Opalith tags, Christian Graze KG, Germany) within a day of emergence from pupae. The colony was provided with ad lib. defrosted pollen (Koppert BV, Berkel en Rodenrijs, The Netherlands) directly into the nest. Workers also collected sucrose solution from artificial flowers in the flight room.

### Artificial Flowers

We used artificial flowers (fig. 1) inspired by designs from earlier studies (Hartling and Plowright 1979; Keasar 2000; Cnaani et al. 2006). The landing platform of each flower was a blue plastic disk (diameter = 60 mm, height = 10 mm) with a narrow hole (diameter = 1 mm) in the center through which bees could extend their proboscises to feed. We chose blue because bumblebees have an innate preference for this color (Raine et al. 2006; Raine and Chittka 2007a), which increased the speed with which they learned to forage on the artificial flowers during pretraining. Underneath the plastic disk was a reservoir (Ø = 30 mm, height = 70 mm, volume = 50 mL) containing sucrose solution (40% [w/w]). Floating in the reservoir was a plastic cylinder (Ø = 15 mm, height = 40 mm) containing a strong neodymium (NIB) magnet, on top of which was a small plastic cup calibrated to contain a fixed amount of sucrose (volume range: 20–45 μL; fig. 1A). The volume

plastic disk with
guiding tube

*plastic cup*

float

*plastic
cylinder*

*magnet*

sucrose
reservoir

electromagnet

*electronic box
containing
integrated circuit*

*remote
control
box*

**A**

**B**
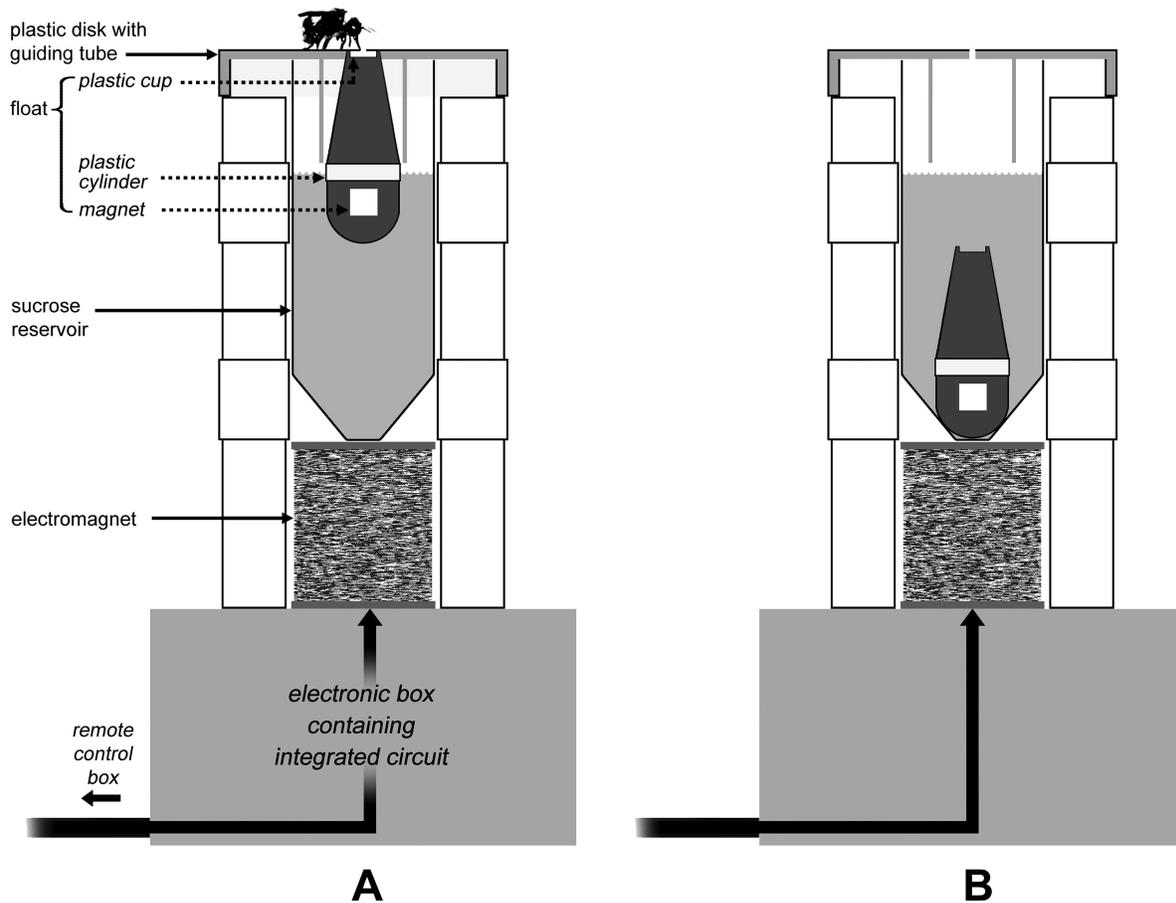
**Figure 1:** Schematic diagrams of a side view of the artificial flower. The cup containing sucrose solution was refilled at a distance by the experimenter by switching on/off the electromagnet using a remote control box. *A*, When the electromagnet is switched off, the cup (containing sucrose solution) floats below the hole in the blue plastic disk through which the bee can feed. *B*, When the electromagnet is switched on, the float is drawn down under the surface of the sucrose solution, which acts to fill the cup. Illustration by S. Blackburn.

of this plastic cup was set to be 25% of the crop capacity of the individual bee being tested. When the electromagnet beneath the reservoir is activated, the float is drawn under the surface of the sucrose solution, which acts to fill the cup (fig. 1*B*). When the electromagnet is switched off, the float bobs up and the cup containing sucrose is guided via a plastic tube just below the hole through which the bee can feed. The bee can obtain only the volume of sucrose solution presented in the cup. The electromagnetic mechanism in all flowers was controlled by the experimenter from a remote control box, reducing any potential interference with the bee's foraging behavior during tests. These flowers can be accessed by a bee equally well from all angles. Consequently, the direction from which they arrive or depart each flower is not constrained.

*Experimental Procedure*

Experiments were performed using four identical flowers, each placed on a wooden support (height = 50 cm). Flowers were placed in four separate positions within the flight room (fig. 2), chosen in order to maximize distances between locations (distance range: 290–960 cm). Each location was uniquely identified with respect to local three-dimensional and two-dimensional landmarks (landmark a: inverted, dark blue plastic flower pot, height = 60 cm, base diameter = 40 cm, top diameter = 25 cm; b: maroon cone [metal frame wrapped in fabric], height = 155 cm, base diameter = 25 cm; c: purple and orange truncated plastic cones, height = 70 cm, base and top diameter = 35 cm, maximum diameter = 50 cm; d: green plastic water container [water butt], height = 95 cm, width and
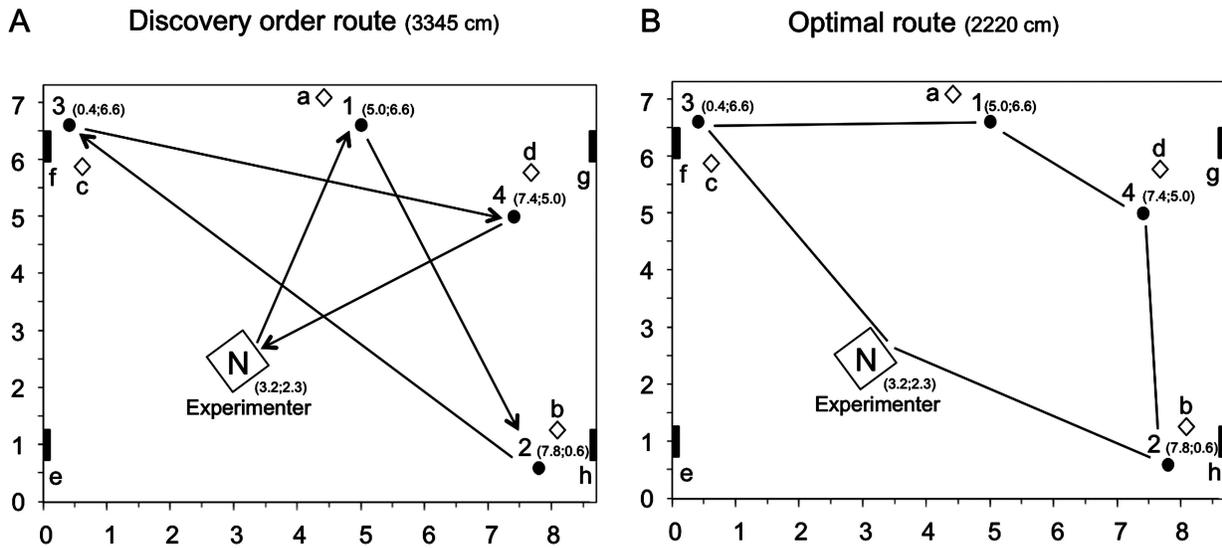
**Figure 2:** Spatial location of flowers (*circles*) and landmarks (*open symbols, solid bars*) in the flight room for configuration 4. Symbols (*a–d*) represent three-dimensional colored landmarks placed on the floor (see details in fig. A1 in the online edition of the *American Naturalist*); solid bars (*e–h*) represent two-dimensional geometric-patterned landmarks attached to the walls (see details in fig. A2); *N* is the nest box. Numbers in parentheses indicate the precise Cartesian coordinates (*x;y*) for each flower and the nest entrance (scale in meters). Bees visiting flowers in the discovery order (*A*; 3,345 cm) fly much farther than if they optimized the route (*B*; 2,220 cm).

depth = 30 cm; landmarks e–h were geometric-patterned posters fixed to the walls [height = 120 cm, width = 85 cm]) to help the bees navigate (see details in figs. A1, A2 in the online edition of the *American Naturalist*).

Before running an experiment, bees were allowed to forage freely on a training flower placed at location 1 (fig. 2), thus giving them the opportunity to associate the blue plastic disk with a sucrose solution reward. During this pretraining phase, the flower reward was set at 20 μL and was refilled ad lib. until the bee returned to the nest. Bees that made at least five foraging bouts within 2 h (i.e., that visited the flower and returned to the nest to deposit the sucrose solution collected) were considered as regular foragers and were allowed to make three additional foraging bouts. The amount of sucrose solution ingested during each of these new foraging bouts was used to estimate the crop capacity of each individual worker (volume range: 120–180 μL). Using this information, we could set the rewards provided by each flower to 25% of an individual's crop capacity during the experiments, so that a worker feeding from all four flowers would fill her crop within a given foraging bout.

Bees were tested individually over two successive days (hereafter days 1 and 2), during which they foraged from flowers in four spatial configurations (fig. A3). Only one bee was observed during each block of 2 days (approximately 8 h on day 1 and 2 h on day 2). During the tests,

the flowers were refilled only after each foraging bout, so that the bee had to visit all four flowers to fill her crop. Scent marks deposited by the test bee when visiting flowers (Witjes and Eltz 2009) were deliberately not removed between successive foraging bouts to favor stabilization of the suboptimal discovery order route to which each forager was trained (see "Route Ontogeny"). Scent mark odors are always strongest at the last visited flower and diminish with time since the flower was last visited. The experimenter remained next to the nest box (acting as a stationary landmark), outside the array but inside the flight room. The behavior of each bee was observed continuously and recorded using ETHOM software (Shih and Mok 2000). We recorded the order (and time) at which the test bee visited each flower, the duration of each foraging bout, and the time spent on each flower. Visiting a flower required the bee to land on the blue disk. The total time spent flying per bout was calculated by subtracting the time spent on each flower from the bout duration. The distance flown by the bee in each bout was calculated a posteriori and represents the minimum distance flown in a straight line between flower locations. At the end of observations, each test bee was frozen (−20°C) and measured (thorax width) to control for body size effects. Between testing bees, we cleaned the landing platform of each flower with ethanol solution (70% [w/w]), so that

the next bee tested could not be influenced by any remaining scent marks deposited by the previous forager.

*Route Ontogeny.* To test whether bees repeatedly visited flowers according to their discovery order or whether they develop new routes with experience, 11 individuals were sequentially tested in the four spatial configurations (fig. A3). Test bees were selected to minimize variations in age and body size (mean ± SE; age: 31.26 ± 4.26 days since eclosion; thorax width: 5.34 ± 0.11 mm). For each new configuration, a new feeding location was introduced into the array. The spacing between the locations was far enough so that neighboring flowers in two different locations (at least 290 cm apart) would be distinguishable to the bees as different patches (the distance between flowers within a patch was always <40 cm). Starting from a single patch containing all four flowers (configuration 1), we progressively moved three of the flowers to new patches so that we ended up with four patches, each containing a single flower (configuration 4). The order in which patches were introduced into the array was chosen to maximize the difference between the lengths of the discovery order and optimal route (figs. 2, A3). On day 1, each bee was observed for 20 foraging bouts per configuration (80 foraging bouts and ~8 h of observation in total). All four spatial configurations were tested in ascending numerical sequences: configuration 1: 1 × four-flower patch at location 1 (discovery order route = optimal route = 970 cm); configuration 2: 2 × two-flower patches at locations 1 and 2 (discovery order route = optimal route = 1,700 cm); configuration 3: 1 × two-flower patch at location 1 and 2 × one-flower patches at locations 2 and 3 (discovery order route = 2,660 cm, optimal route = 2,185 cm); configuration 4: 4 × one-flower patches at locations 1–4 (discovery order route = 3,345 cm, optimal route = 2,220 cm).

*Overnight Memory Retention.* To test whether bees have a long-term memory of their foraging routes or whether they establish completely new routes after extended breaks, 10 individuals were retested on day 2 for 20 foraging bouts in configuration 4 (fig. 2) after an overnight interruption of foraging.

### Data Analysis

Data were analyzed using R statistical software (ver. 2.9.0; R Development Core Team 2009). Tested bees visited all four flowers of the array in 95% of the 1,080 foraging bouts observed in total (see detailed sequences in fig. A4). Since the frequencies of incomplete foraging bouts were low and equally distributed among the four configurations ($\chi^2$ test; configuration 1: 0.91%; configuration 2: 3.64%;

configuration 3: 3.18%; configuration 4: day 1, 0.91%; day 2, 1.50%; $\chi^2_4 = 7.55$, $P = .102$), we excluded these observations from the data set to facilitate further analyses.

*Foraging Performance.* To determine the effect of experience on individual foraging performance (number of revisits to the same flower, total flight duration, total flight distance) in each spatial configuration, we used generalized linear mixed models (GLMMs). Normally distributed data (confirmed by a Shapiro-Wilks normality test) were analyzed using an identity-link function, and significance of terms was assessed using $F$ ratios. Data with a Poisson distribution were analyzed using a log-link function, and significance of terms was assessed using $\chi^2$ Wald statistics. In all models, the number of foraging bouts the bee completed was included as a continuous variable (experience effect) and the identity of individual bees as a random effect (individual effect). Direct comparisons between observations made on days 1 and 2 on the same individuals were performed using paired $t$-tests or Wilcoxon tests.

*Route Repeatability.* To quantify the repeatability of routes followed by each individual, we calculated a similarity index (SI) for pairs of flower visitation sequences, using a simple technique derived for aligning DNA sequences (Waterman and Jones 1990). This procedure takes into account insertions, deletions, and substitutions to any primary sequence and allows us to identify changes between two successive foraging sequences (including any revisits to the same flower), starting and ending at the nest (Thomson et al. 1997). The algorithm is best understood by envisioning the two sequences written out as the row and column headings of an $n \times m$ matrix, where $n$ and $m$ are the lengths of the two sequences. The elements of the matrix are scored as 1 if the row and column headings match or as 0 if the headings differ. Then, dummy rows and columns are inserted to put as many of the 1's as possible on the principal diagonal. To obtain the SI, we divided the number of matches on the diagonal of this expanded matrix by the total number of cells along the diagonal (see examples in fig. A5). The SI ranges between 0 (the visitation sequences are completely different) and 1 (the visitation sequences are identical). We then averaged SI values to give a mean value representing a moving average across bins of five consecutive bouts (1–5, 2–6, 3–7, etc.). Averaging data in this way removed the effect of variation between pairs of bouts and revealed gradual changes in the bee's tendency to trapline (Saleh and Chittka 2007).

To determine whether bees showed a greater tendency to trapline than expected by chance, we compared SIs calculated from our observations with those calculated from sequences from a null model (nontraplining hy-

**Table 1:** Comparison of route use across spatial configurations (1–4)

| | Configuration 1 (day 1) | Configuration 2 (day 1) | Configuration 3 (day 1) | Configuration 4 (day 1) | $P^a$ | Configuration 4 (day 2) | $P^b$ |
|---|---|---|---|---|---|---|---|
| Total no. routes explored | 7.45 ± .6 | 6.09 ± .44 | 6.09 ± .25 | 7.45 ± .53 | .055[c] | 6.78 ± .82 | .225[d] |
| Total no. significant routes | 2.55 ± .21 | 2.18 ± .20 | 2.64 ± .20 | 2.73 ± .19 | .641[e] | 2.20 ± .20 | .120[f] |
| Route use: | | | | | | | |
|   Primary (%) | 38.56 ± 4.35 | 47.05 ± 2.87 | 43.62 ± 3.83 | 37.15 ± 2.16 | .772[c] | 47.12 ± 6.29 | .135[d] |
|   Secondary (%) | 22.06 ± 1.47 | 24.99 ± 1.64 | 19.48 ± .91 | 21.53 ± 2.29 | .294[e] | 20.33 ± 1.84 | .906[f] |
|   Discovery order (%) | … | 79.19 ± 7.61 | 27.25 ± 6.48 | 2.72 ± 1.56 | <.001[c] | 1.50 ± 1.07 | .434[d] |
|   Optimal (%) | … | 94.52 ± 3.26 | 49.89 ± 5.10 | 37.68 ± 2.90 | <.001[c] | 30.01 ± 7.02 | .260[d] |

Note: Means ± SE are given for 11 bees (day 1) or 10 bees (day 2).

[a] Comparison between configurations 1–4 in day 1.

[b] Comparison between configuration 4 in days 1 and 2.

[c] GLMM with Gaussian error structure (categorical variable: spatial configuration; random factor: individual).

[d] Paired *t*-test.

[e] GLMM with Poisson error structure (categorical variable: spatial configuration; random factor: individual).

[f] Wilcoxon test.

pothesis). We generated 2,500 random visitation sequences according to the two following constraints: (1) the individual makes random visits until each of the four flowers has been visited at least once and (2) the maximum number of visits (including revisits) to flowers cannot exceed 16 (98% of our observations). As before, we calculated SIs for pairs of successive sequences and analyzed average indices for bins of five bouts. Because 95% of the 500 randomly generated average SIs fall below a threshold of 0.43, we defined an individual bee's SI as nonrandom (at the 5% level) if it exceeded this threshold. While randomness of movements within a range of four to 16 flower visits is perhaps a simplistic assumption of how bees might move in the absence of traplining, such a model has the virtue of being free from ad hoc assumptions about alternative strategies that bees might use.

To determine the influence of discovery order of flower patches on the overall spatial geometry of routes, we reexamined all sequences, taking into account only the first visit to each flower. We deliberately excluded all the revisits to the same flower, since the majority (66.14% of the revisits, $n = 2,156$) were returns to the flower just visited (intrapatch revisits), not to locations in different parts of the flight arena (interpatch revisits). Such revisits are typically observed in inexperienced bees. Possibly they act to consolidate spatial memory of flowers, and the frequency with which they occur drops rapidly with experience (Saleh and Chittka 2007; this study). Consequently, the occurrence of revisits does not appear to influence the overall sequential activation of spatial memories. Assuming that there are 24 different possible routes to visit all the flowers once in a four-flower array ($4! = 4 \times 3 \times 2 \times 1$), we used multinomial tests with a random probability of 0.042 (1/24) to explore each route per foraging bout. Because each bee was tested for 20 foraging bouts in each configuration, routes that were used more than three times in

the same configuration by a single bee were considered to be used significantly more often than expected by chance (at the 5% level). Comparisons of the routes used by bees across spatial configurations were performed using GLMMs, including configuration as a categorical variable (configuration effect) and individual as a random effect (individual effect).

## Results

### Spatial Geometry of Routes

Detailed analysis of the four-flower visitation sequences (ignoring revisits to the same flower) indicates that each bee explored an average of 6.77 ± 0.25 (mean ± SE) routes per spatial configuration on day 1 ($n = 11$ bees). This observation was consistent throughout the four configurations (table 1; see detailed sequences in fig. A4). Among these routes, the bees used a set of two or three sequences more often than expected by chance (multinomial tests, $P < .05$). The most frequently used route (primary route) was repeated in approximately 40% of the foraging bouts and the secondary routes in 20% of the foraging bouts (table 1). Focusing on the sequence in which bees visited patches (ignoring the order of flower visitation within patches) clearly demonstrates that bees tested in configuration 2 (where the discovery order sequence coincided with the shortest possible route) used the discovery order sequence in the majority of their foraging bouts (table 1). However, when tested in configurations where the discovery order sequence was much longer than the optimal route (22% longer in configuration 3, 51% longer in configuration 4), they drastically reduced their use of the discovery order sequence. This abandonment of the discovery order route coincided with a tendency to develop more optimal solutions, thereby

using shortest possible routes in about 40% of their foraging bouts in configurations 3 and 4 (table 1). Overall, 80% of the bees adopted an optimal sequence as their primary route in the final array by turning either clockwise or counterclockwise (configuration 4; fig. 3). Therefore, bee foraging sequences do not simply reflect the order in which patches were initially encountered but clearly result from sequential readjustments of preexisting routes after the incorporation of new patches.

Comparison of the four-flower visitation sequences (ignoring revisits to the same flower) between days 1 and 2 reveals that each bee persisted in exploring approximately seven different routes after the overnight break, using two or three routes significantly more often than expected by chance (table 1; fig. A4). The primary route developed during day 1 was conserved by 70%—and secondary routes by 30%—of bees tested on day 2 ($n = 10$). Despite this strong consistency in traplines, all the bees continued to develop alternative solutions; notably, they all explored at least one entirely novel route (i.e., a route not observed on day 1) in 23.44% ± 4.90% (mean ± SE) of their foraging bouts, suggesting that they persisted in scanning for any environmental changes. Visitation sequences of patches clearly indicate that, as on day 1, bees used the discovery order route in only 2% of their foraging bouts and never adopted it as a primary route (table 1). Instead, they persistently tended to minimize their total flight distance by using the shortest possible routes in approximately 30% of their foraging bouts. Forty percent of the bees consistently used an optimal sequence as their primary route (fig. 3).

### Route Ontogeny

In all the four spatial configurations tested in day 1, complete flower visitation sequences (including revisits to the same flower) became more similar with experience. Comparing SIs calculated from experimental data and randomly generated sequences indicates that bees always reached significantly higher values than expected by chance at the end of the 20 foraging bouts (fig. 4). While SI values were significantly higher than chance after the first bin of five foraging bouts in configuration 1, they only reached significance later during the 10 first bins of five foraging bouts in configurations 2–4. Bees thus developed repeatable routes faster in the simple one-patch array than in the complex multipatch ones. This general increase in route repeatability was accompanied by a significant reduction in the number of revisits to the same flower within foraging bouts. This is true for both immediate revisits to flowers from the same patch but also for revisits to a previously visited flower in a different patch (fig. 5). In doing so, bees significantly reduced their total flight du-

ration (GLMM, experience effect; configuration 1: $\chi_1^2 = 2{,}475.53$, $P < .01$; configuration 2: $\chi_1^2 = 2{,}861.99$, $P < .01$; configuration 3: $\chi_1^2 = 1{,}737.48$, $P < .01$; configuration 4: $\chi_1^2 = 1{,}173.98$, $P < .01$) as well as their total flight distance per foraging bout (fig. 6). Thus, in all four spatial configurations, bee foraging performance improved with experience and saturated near optimality.

### Overnight Memory Retention

Bees retested on the day after training started to forage with more repeatable sequences (complete flower visitation sequences including revisits to a same flower) than expected by chance (fig. 4). Average SI for their first five foraging bouts at the beginning of day 2 were significantly lower than at the end of testing on day 1 (table 2). However, bees did not show any significant increase in route repeatability after the 20 foraging bouts on day 2, compared with their performance after completing 20 foraging bouts on configuration 4 during day 1 (table 2). Numbers of revisits to the same flower were significantly higher at the beginning of day 2 than at the end of day 1, whether they immediately revisited flowers in the same patch or in another patch (table 2; fig. 5). The number of revisits then progressively decreased with experience to reach values similar to those observed at the end of day 1. Similar observations were made for flight durations and flight distances (table 2). Bees flew for longer and traveled greater distances at the beginning of day 2 than at the end of day 1. As for revisits, both indicators of foraging performance decreased as bees gained experience (GLMM, experience effect, flight duration; $\chi_1^2 = 305.47$, $P < .01$; for flight distances, see fig. 6) and reached values similar to those observed at the end of day 1. Altogether, these results demonstrate that bees remembered the main characteristics of the spatial configuration they experienced on day 1. They also suggest either that (overnight) memory was imperfect on the next day or that bees "deliberately" depart from their memorized routes after an overnight break, perhaps to explore new solutions or to allow the inclusion of novel flowers at new locations if these should occur.

### Discussion

We found that bees adjust their foraging routes after the discovery of new flowers, allowing them to develop optimal routes rather than simply following the suboptimal flower discovery order. Although their foraging performance declines to some extent after an overnight interruption, bees continue to prioritize routes they experienced on day 1 while retaining the ability to explore completely new flight paths. We discuss how maintaining some degree of route flexibility in conjunction with high
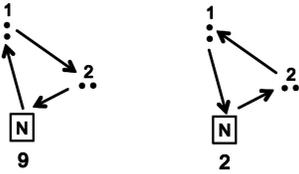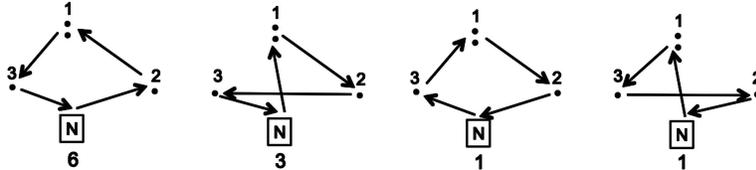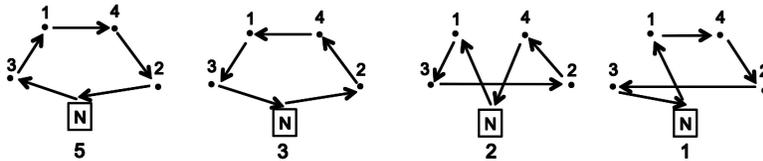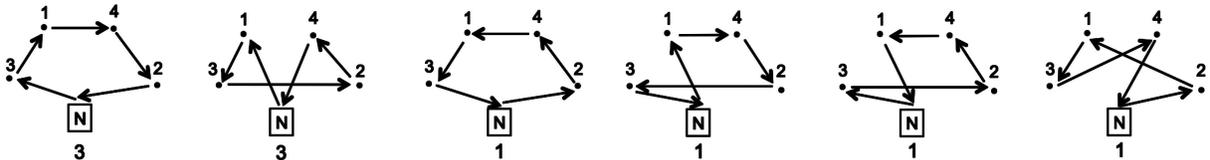
**Figure 3:** Patch visitation sequences (excluding revisits). Diagrams represent patch visitation sequences mostly used by bees for spatial configurations 2–4. Circles represent flowers, numbers (*1–4*) are patch locations, *N* is the nest box, and arrows indicate the direction of bee movements. The number of bees that adopted the corresponding sequence in the majority of their foraging bouts is indicated below each diagram.

levels of fidelity to learned foraging circuits could allow traplining animals to track environmental changes and develop efficient solutions to complex dynamic routing problems.

*Readjustment of Traplines after Discovery of New Patches*

Although many animals have been shown to trapline when visiting familiar resources (e.g., bats [Lemke 1984], bees [Thomson et al. 1997], butterflies [Gilbert 1980], hummingbirds [Gill 1988], monkeys [Janson 1998], and rats [Reid and Reid 2005]), previous studies have typically taken snapshots of individuals' behavior when their routes have already become well established; therefore, the history of how such routes developed is irretrievable. To our knowledge, our study is the first in which new foraging

locations have been sequentially introduced into animals' familiar environments, allowing us to trace in real time how individuals integrate information from history and exploration to form functional routes linking multiple locations. While observing euglossine bees flying long distances between plants and linking them in a repeatable but suboptimal order, Janzen (1971) suggested that traplining animals would invariably visit resources (or resource patches) in their original discovery order. Our results provide clear evidence that animal traplines can develop independently of the order in which resources are encountered but result from more complex navigational strategies, allowing them to reduce their overall travel distance with experience. In this study, the discovery order route was used only when it coincided with the shortest possible route to link the four flowers in the array (configurations
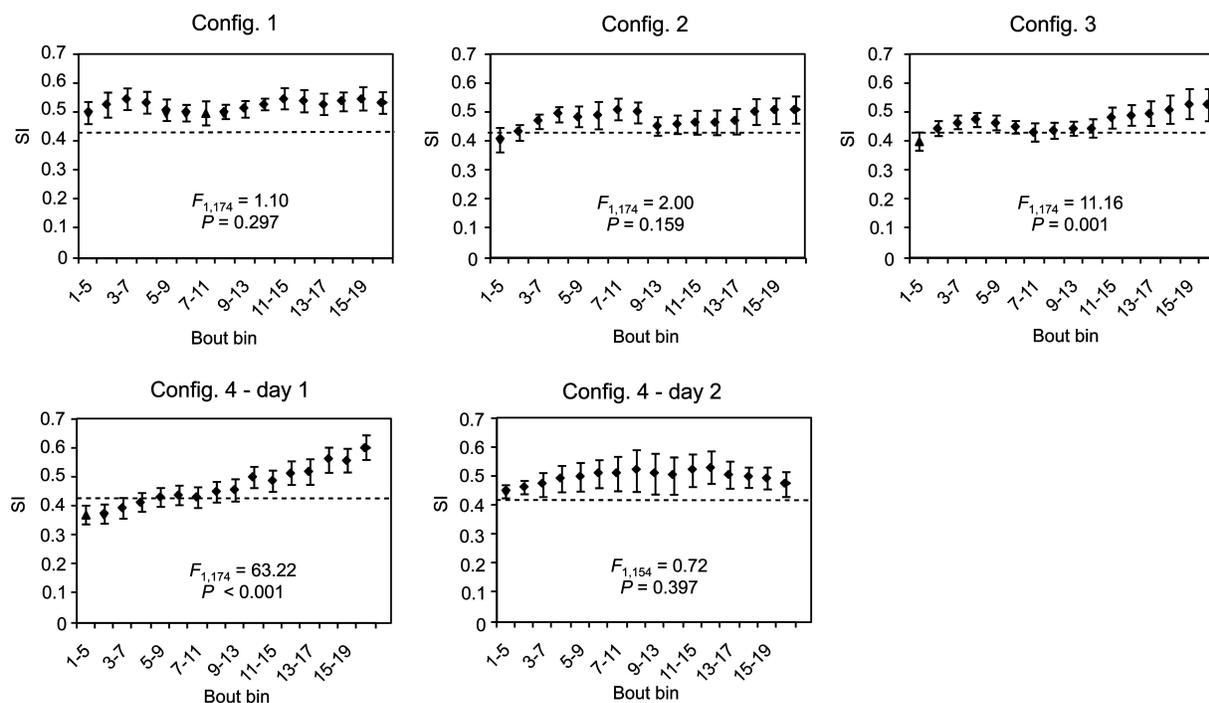
**Figure 4:** Moving average (mean ± SE) similarity indices (SIs) of traplining bees for each configuration. Calculations of SIs take into account revisits to the same flower. The higher the SI value, the greater the similarity between bouts (i.e., tendency to trapline). Bee SIs were compared with 500 indices produced by randomly generated sequences. Because 95% of the SIs from the randomly generated sequences fall below the threshold value of 0.43 (*dashed horizontal line*), when a bee's SI exceeds this value, it is considered significantly different from chance (at the 5% level). *P* values: GLMM with Gaussian error structure (dependent variable: SI; independent variable: bout bin; random factor: individual).

1 and 2) but was progressively abandoned when the discrepancy with the optimal route increased (configurations 3 and 4). After the discovery of a new rewarding patch, bees rearranged their visitation sequence and tended to reduce their total flight distance by selecting the shortest possible circuit. Eighty percent of bees converged on using an optimal route as a trapline (primary route), turning either clockwise or counterclockwise, in the final (four-patch) spatial array. This is perhaps more impressive when we consider that the bees appear to optimize their flight routes despite the presence of both conspicuous landmarks (placed near flowers to facilitate the retrieval of landmark sequences in the entrainment order) and scent marks that should have favored the stabilization of the suboptimal—discovery order—route.

Despite frequent suggestions of the utility of distance optimization by traplining animals in the theoretical literature (Anderson 1983), this behavioral phenomenon has not been clearly demonstrated before. Indeed, the few studies that provide geometrical details of routes indicate that traplining animals follow suboptimal circuits both in the laboratory (Bures et al. 1992; Saleh and Chittka 2007) and in the wild (Janzen 1971; Noser and Byrne 2010). Our

results suggest that such a failure to optimize travel distance could be related to the scale of observation. At small spatial scales—for example, within a patch of aggregated resources, where the differential cost of traveling alternative routes is low (or perhaps negligible)—animals preferentially rely on simple movement rules, such as linking nearest-neighbor locations (e.g., Bures et al. 1992; Cramer and Gallistel 1997; Ohashi et al. 2007), making short trips after encountering highly rewarding resources (e.g., Chittka et al. 1997; Raine and Chittka 2007*b*), or continuing to move in a constant direction (e.g., Pyke and Cartar 1992), even if they lead to suboptimal pathways. Using such simple movement rules to link visually detectable feeding locations increases foraging efficiency by minimizing the travel time and also reduces the chances of revisiting a patch recently depleted by the same individual (Pyke 1978). However, at larger spatial scales—for example, movements between scattered resource patches, where the costs of traveling different routes become magnified—we would expect animals to cease relying solely on simple rules of thumb and instead tend toward strategies that optimize their patch visitation sequence, thereby developing more efficient traplines. Our results therefore
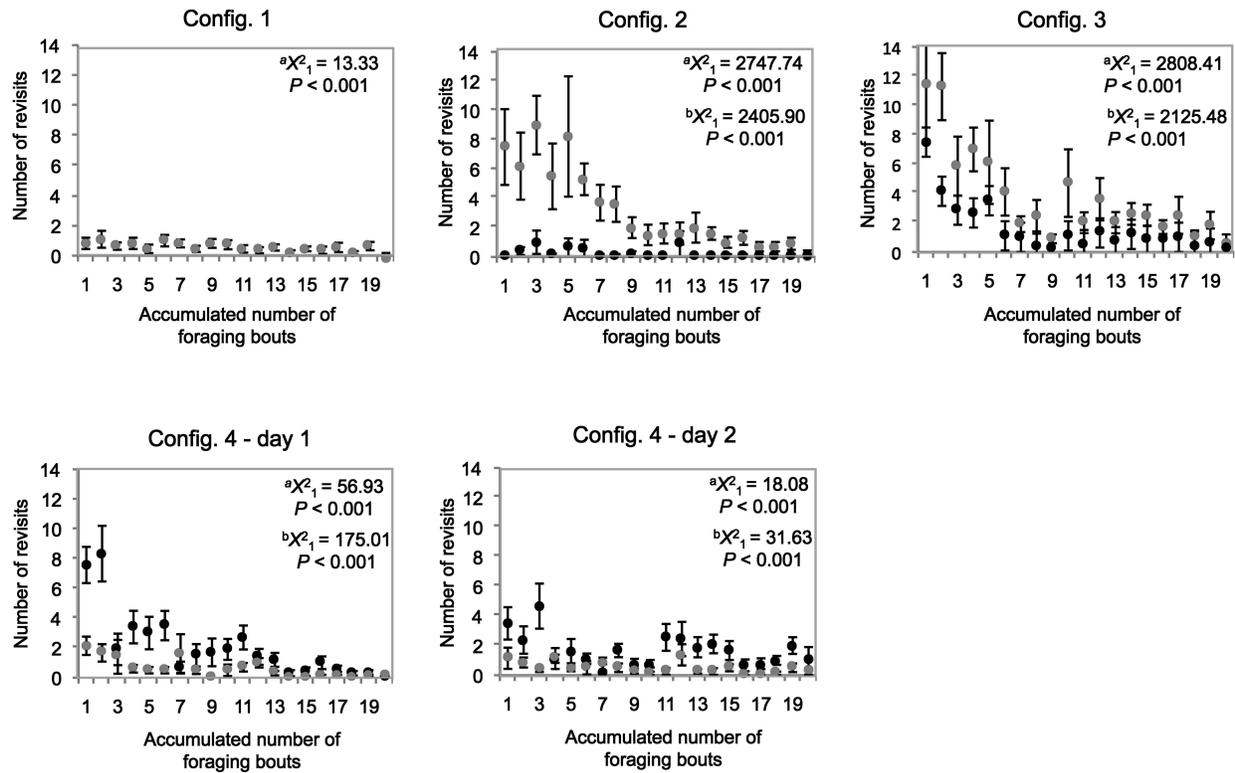
**Figure 5:** Average (mean ± SE) number of revisits to flowers of the same patch (*gray circles*) and to flowers of different patches (*black circles*) per foraging bout in relation to the number of accumulated foraging bouts, for each configuration. Bees' tendency to revisit flowers dropped rapidly with increasing experience. *P* values: GLMM with Poisson error structure (dependent variable: number of revisits; independent variable: number of foraging bouts completed; random factor: individual). *a*, Intrapatch revisits. *b*, Interpatch revisits.

highlight the need to consider trapline foraging as a strategy potentially involving different decision rules at a range of spatial scales. However, it is also possible that additional factors could explain the apparent absence of travel distance optimization in other studies. In particular, further experiments should explore the influence of prioritizing the most productive resources (Williams and Thomson 1998; Garrison and Gass 1999; Cunningham and Janson 2007), avoiding competitors (Temeles et al. 2006; Ohashi et al. 2008), or simply following group members (Janson 1998) on the spatial geometry of traplines.

### Maintenance of Route Flexibility after the Establishment of Traplines

Studies of bats (Lemke 1984), bees (Saleh and Chittka 2007), hummingbirds (Garrison and Gass 1999), and primates (Noser and Byrne 2010) suggest that when stable routes are established, individuals do not simply focus on a single solution but rather alternate between different options. Our detailed analysis of the geometry of routes

used by bees provides clear support for these observations and indicates that each individual developed a set of idiosyncratic routes involving a primary route (or trapline) used in ∼40% of all foraging bouts and two or three secondary (less frequently used) routes in ∼15% of bouts. On the next day (day 2), bees remembered the spatial configuration of feeder locations they experienced the previous day (day 1) and reused learned routes consistently, even though their foraging performance was markedly poorer than at the end of training the previous day. This decrease in foraging performance (after an overnight break) may be to some extent due to passive memory decay and interpreted as imperfect memory retention, but it is also conceivable that bees retained their memories intact overnight and chose not to use them on the first few foraging bouts of the following day. Interestingly, on day 2 of our experiments, all bees explored routes they had not followed on the previous day in approximately 25% of their foraging bouts. Such a departure from memorized information may be adaptive and allow individual foragers to react quickly to changes in resource profitability. If so,
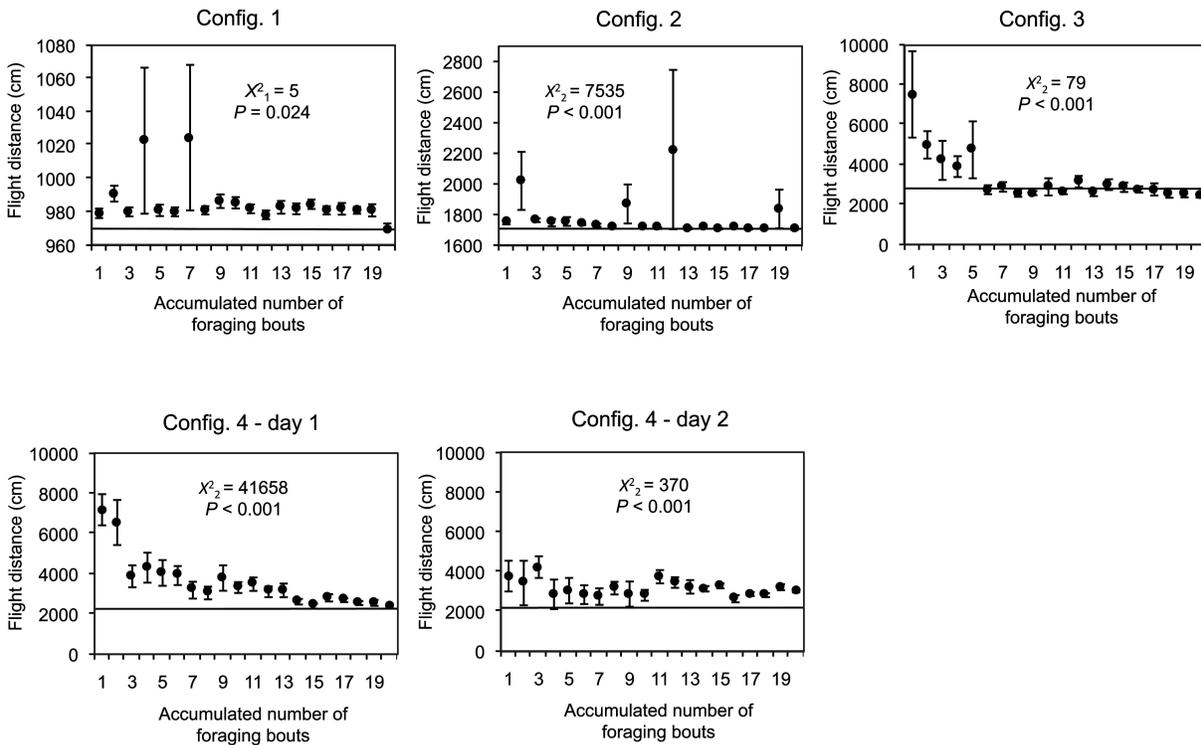
**Figure 6:** Average flight distances (mean ± SE) per foraging bout in relation to the number of accumulated foraging bouts for each configuration. Bee performance approached saturation level—that is, the optimal route (*solid horizontal line*)—as they gained experience in the array. *P* values: GLMM with Poisson error structure (dependent variable: flight distance; independent variable: number of foraging bouts completed; random factor: individual).

we would expect similar switches between high route fidelity and exploration phases to occur in other traplining animals. In maintaining low levels of route flexibility, individuals may optimize spatial solutions in the face of environmental changes by not sticking entirely to established traplines but instead exploring alternative solutions to incorporate or ignore novel patches (Ohashi and Thomson 2005).

## Trapline Foraging and Dynamic Traveling Salesman Problems

Traplining animals are faced with complex routing problems analogous to the well-known traveling salesman problem (Applegate et al. 2006; Gutin and Punnen 2006). Rather than calculating and comparing all possible alternative paths, it is generally assumed that animals rely on simple heuristics coupled with some form of spatial memory that gives a reasonable approximation to the optimal solution with relatively little cognitive effort (Anderson 1983; Cramer and Gallistel 1997). Our findings suggest that traplining animals can find (or approach) optimal

solutions to dynamic traveling salesman problems (variations of the classic problem where availability of sites changes over time) simply by adjusting their routes by trial and error in response to environmental changes. Indeed, individuals may not need to build a complex mental spatial representation to navigate efficiently (Garber and Dolins 2010) but could simply encode spatial information topologically according to a route-based system, where space is represented as a large number of segments grouped together in sequence to form routes (Poucet 1993). Thus, when it recognizes a familiar cue (landmark or view) along a route, an animal may perform an action(s) that it has associated with that cue, such as making a turn or moving in a particular compass direction. Some extent of behavioral flexibility would enable an animal to deviate from established routes, but the potential for route innovation could be at least partially constrained (e.g., by interference with learned associations/instructions that may not be easy to ignore or replace). In this way, animals would be able to select new optimal solutions by comparing the length of the different routes they previously explored.

Although this model is a simplistic explanation to de-

**Table 2:** Comparison of foraging performance after the overnight break (configuration 4)

| | End of day 1 | Start of day 2 | End of day 2 | $P$[a] | $P$[b] |
|---|---|---|---|---|---|
| Similarity indices of sequences | .65 ± .05 | .49 ± .02 | .52 ± .04 | .005[c] | .024[c] |
| No. revisits to flowers in the same patch | .30 ± .30 | 2 ± .75 | .40 ± .26 | .035[d] | >.99[d] |
| No. revisits to flowers in a different patch | 0 | 3 ± 1.10 | 1.7 ± .90 | .006[d] | .054[d] |
| Flight duration (s) | 25.39 ± 4.61 | 57.88 ± 8.56 | 22.20 ± 3.66 | .004[d] | .554[d] |
| Flight distance (cm) | 2,322 ± 69.85 | 3,778.89 ± 589.54 | 3,029.5 ± 389.18 | .030[d] | .058[d] |

Note: Means ± SE are given for the 10 bees tested on days 1 and 2.

[a] Comparison between values at the end of day 1 and values at the start of day 2.

[b] Comparison between values at the end of day 1 and values at the end of day 2.

[c] Paired *t*-test.

[d] Wilcoxon test.

scribe how traplining animals may encode spatial information and develop functional routes, it is certainly an interesting theoretical approach to explore new solutions for traveling salesman problems in a dynamic environment. To date, the most powerful algorithms have been inspired by swarm intelligence theory (the ability of animals to solve complex problems collectively) on the basis of the metaphor of ant colony foraging behavior (Dorigo et al. 1999; Bonabeau et al. 2000). In these models, artificial ants lay artificial pheromone on the route sections they use during their tour around the sites in quantities proportional to the quality of the tour; this means that route sections from good solutions end up with more pheromone. Because each ant tends to choose route sections with the most pheromone, this selection process amplifies previously reinforced sections and tends to lead to the emergence of an improved global solution to the routing problem. Similar to ant colony foraging behavior, we believe that there is great potential for the mechanisms of route optimization by traplining animals to inspire computer scientists to develop evolutionary algorithms. Sequential adjustment of routes in relation to past experience is a parsimonious way to keep track of the optimization steps. In case of changes in the spatial configuration of sites, the algorithm does not need to restart the entire procedure; it needs only to adapt the results to the new conditions.

## Conclusions

Although the adaptive value of trapline foraging has received much attention in recent years, we still know comparatively little about how animals encode spatial information and develop functional routes to link multiple locations. Detailed analyses of the ontogeny and geometry of traplines are necessary to refine our understanding of this important and taxonomically widespread animal foraging strategy, by probing the limits of spatial navigation and exploring efficient solutions to traveling salesman problems. Expanding this approach to the field is also fundamental to clarifying the influence of traplining animals in shaping the structure of landscapes via pollination and seed dispersal.

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Bumblebee (*Bombus terrestris*) queen collecting pollen. Photograph by Nigel E. Raine.